ABSTRACT

Erythromycin (ERY) is a macrolide antibiotic that has similar antimicrobial spectrum to penicillin and it is many used, particular in the treatment of patients who are allergic to penicillin. A simple, efficient and low-cost method based on matrix solid-phase dispersion (MSPD) microextraction was developed for the determination of erythromycin in environmental samples. In this work, Fe₃O₄ nanoparticles were adsorbents and spectrophotometric methods have been developed and validated for the isolation and pre-concentration of erythromycin form aquatic.

The experimental conditions for the extraction, including Fe₃O₄ nanoparticle dosage, amount of the salt, the extraction time, and pH were investigated and optimized. Under the optimized conditions, a linear range of 10 - 50000µgL⁻¹ with R² = 0.9551 and detection limit of 17 µg L⁻¹ were obtained for target analyte. The method was successfully used for the extraction of ERY from aqueous samples with relative recoveries amount of 94.8 (RSDs =4.4). These results corroborate that this method is low cost, conveniently fast and prepare reliable results in order to be used in detection of erythromycin from aqueous samples.

Keywords: Erythromycin, spectrophotometry, nanoparticles, antibiotic

INTRODUCTION

Erythromycin, produced by Saccharopolyspora erythreas (formerly known as Streptomyces erythraeus) during fermentation process, is abroad_spectrum macrolide antibiotic consisting of 14 membered lactone ring with ten asymmetric centers and two sugar molecules (L-cladinose and D-desoamine). In fermentation process several related substances of erythromycin can beformed. Even though erythromycin A is the main component of commercially available erythromycin, some structurally and chemically similar analogs, especially erythromycin B and erythromycin C, are presented in small amount [1–4].

The structures of erythromycin A and its related substances are shown in Fig. 1. Up to now, there have been various methods reported for the determination of erythromycin in different sources by several methods including high-performance liquid chromatography [5–7], liquid chromatography–mass spectroscopy [8–10], liquid chromatography–tandem mass spectroscopy [11–14], capillary electrophoresis chromatography [15,16], near infrared reflectance spectroscopy [17], ultraviolet [18] and electrochemical detection [19–21]. Although these analysis methods are successfully applicable for erythromycin, some of these methods are also complicated, expensive, time consuming and laborious [3,21]. Therefore , it is necessary for us to find a simple, low cost, selective and quick response method which serves as an alternative detection method for erythromycin.
The application of nanomaterials to solve environmental problems has received significant attention in recent years. One of the most interesting and promising fields is the research on metal-oxide nanoparticles. Magnetite nanoparticles have shown great potential for many nanotechnology applications, including effective adsorbents for removal of undesirable contaminants in water treatment [22]. This nano-adsorbent has a high surface area and a small particle size. Especially, the superior magnetic property of these adsorbents makes them to be simply recovered by magnetic separation after adsorption or regeneration, which overcomes the drawback of separation difficulty of usual adsorbents [23]. The application of magnetic nanoparticles as an adsorbent has received considerable attention in recent years [24–27]. There are many methods to prepare Fe3O4 nanoparticles such as energy milling [28], reducing [29], ultrasonic assisted impregnation [30], Co-precipitation [31] method and using Tridax procumbens leaf extract [32].

This current study is the applicability of solid phase micro extraction using Fe3O4 nanoparticles as sorbent for the preconcentration of ERY aqueous samples following their subsequent quantification with spectrophotometry.

MATERIALS AND METHODS

Experimental
All absorption spectra were carried out using shimadzu recording spectrophotometer UV 160 equipped with matched 1-cm quartz cells. The ultrasonic processor apparatus model UP-100H (Hielscher-Germany) was used. The FT-IR instrument that was utilized for recording the infrared spectrum was M-500 Fast-Scan IR spectrometer (Buck Scientific, East Norwalk, CT 06855, USA). The transmission electron microscopic analysis that used was a Jeol 2010 instrument with an accelerating voltage of 200 kV. X-ray powder diffraction patterns of the products were recorded on a Shimadzu XRD-6000 x-ray diffractometer at a scanning rate of 0.05°s⁻¹ in the 2θ range from 10 to 80° with high-intensity CuKα radiation (λ=0.154178 nm).

Reagents
Analytical reagent grade chemicals and deionized water were used. Standard stock solution of ERY1000 mg L⁻¹ in methanol was prepared. this solution was kept in a refrigerator. Working solutions of lower concentrations were prepared daily by appropriate dilution of the standard solution in deionized water.

Synthesis of Fe3O4 nanoparticles
The magnetic Fe3O4 NPs were prepared by the chemical co-precipitation method. Briefly, 10.3 g of FeCl3·6H2O, 4.1 g FeCl2·4H2O, and 1.5 mL HCl (conc.) were dissolved in 30 mL water under a N2 stream. This solution was added drop-wise into 250 mL of sodium hydroxide (1.8 M) under a nitrogen atmosphere and vigorously stirred for 40 min. The resulting black precipitate was separated with a magnet and washed several times with degassed water; it was stored in 500 mL degassed water under a nitrogen atmosphere.
µ-SPE procedures
For this purpose, 10 mg Fe$_3$O$_4$ nanoparticles were added to 10 ml solution of 2 mg L$^{-1}$ ERY and was mixed on a shaker by a definite rate. The solution was centrifuged for 5 minutes in 5000 rpm. In this step, the nanoparticles was deposited and the tap solution were removed. Then 0.5 ml of methanol was added to Fe$_3$O$_4$ nanoparticles, after mixing and centrifuging (5000 rpm, 5 min), the nanoparticles was deposited and the enriched methanol of analysis were transferred to the spectrophotometric cell. The adsorbance before and after adsorption of the ERY were measured.

Pre-concentrate factor = $A_2/A_1$
$A_1$ and $A_2$ are the adsorption before and after extraction respectively.

RESULTS AND DISCUSSION

Absorption and FT-IR spectrums of erythromycin
Fig. 2 shows the UV-VIS FT-IR spectrums of erythromycin (30 mg L$^{-1}$ in methanol). The characteristic bands can be observed: OH at 3473.97 cm$^{-1}$; and C=O at 1732.50 cm$^{-1}$.

Fig. 2: FT-IR (a) and UV-VIS (b) spectrums of erythromycin
The absorption spectra of the erythromycin has maximum absorbance at 228 nm.

Fig. 3: FT-IR spectrum of Fe$_3$O$_4$ nanoparticles
FT-IR spectrum and characterization of Fe₃O₄ nanoparticles

Fig. 3 has shown the FT-IR spectra of Fe₃O₄ nanoparticles. The broad feature in the range 3,440–3,221 cm⁻¹ was due to O–H stretch (m1), which corresponds to the hydroxyl groups attached by the hydrogen bonds to the iron oxide surface, and also the water molecules chemically adsorbed to the magnetic particle surface. The transmittance waveband from 636 to 574 cm⁻¹, which corresponds to the metal–oxygen bonds, is considered as an indication of the ferrite formation.

The morphology of the product is examined by transmission electron microscopy (TEM) images of Fe₃O₄ nanostructures (fig. 4), the average particle size is about 25 nm, according with the calculated value. XRD pattern has shown in fig 5. The diffraction peaks can be assigned to the planes of inverse cubic spinel structured Fe₃O₄ (JCPDS no. 19-0629), broad peaks indicate the nanocrystalline nature of the prepared MoO2salpr/SCMNP.

The pre-concentration factor of ERY on Fe₃O₄ nanoparticles was studied at different adsorbent amount (5.0–20 mg), ERY concentration of 2 mg L⁻¹, pH 8.0 and agitation time was 5 min. Figure 6 shows that by increasing the amount
of adsorbent up to 10.0 mg, the adsorption of ERY increased. This could be explained by the fact that more adsorbent became available for adsorbing the ERY molecules. Further addition of the adsorbent did not show any significant change in Concentrate factor of ERY. Thus, 10.0 mg of the nanoparticles was chosen as the optimum amount for performing the following steps of the optimization procedure.

**Effect of pH of aqueous sample**

The pH of sample solution could affect the extraction efficiency of target analytes and be considered as an important parameter in the µ-SPE procedure. The effect of sample pH was optimized over the range of 3–10 by adding the appropriate hydrochloric acid or sodium hydroxide solution to aqueous samples. The best extraction efficiencies were obtained when the PH was 8. In the pH above or below than 8, the extraction efficiency decreased rapidly.

In acidic medium – which is presented in the stomach – erythromycin is unstable, forming degradation products which exhibit lower antimicrobial activity [34,35]. Erythromycin A is relatively stable in the pH range between 4 and 10 (with degradation of less than 5% in the incubation period of 48 hours), while at pH ≤ 3.0 degradation is rapid with pseudo-first order rate constants of 2.36 × 10^-1 and 1.30 × 10^-2 for pH 2.0 and pH 3.0, respectively [36]. As a result, pH 8 was adopted as the optimized in subsequent experiments (fig 7).

![Fig. 7: Effect of pH of ERY solutions on extraction efficiency of ERY. Experimental conditions: Fe3O4 amount of 10.0 mg; initial ERY concentrations of 2.0 mg L^-1; extraction time of 5 min and percent of NaCl 10% w/v](image)

**Effect of extraction time**

The extraction time has a significant impact on the extraction efficiency of tested analytes. As is knowing to all, the sample needs to be completely dispersed in the adsorbent. Hence, different extraction times (2 to 20 min) were evaluated in this study (fig 8). The extraction efficiency of the target analytes were obviously enhanced on the whole with the increase of the extraction time in 5 min. After this time the peak of the test compounds was decreased. The cause for this phenomenon may be that the completed extraction equilibrium of target analytes in adsorbent might be reached in 5 min. Accordingly, 5 min was selected as the optimum extraction time.

![Fig. 8: Effect of extraction time on extraction efficiency of ERY. Experimental conditions: Fe3O4 amount of 10.0 mg; initial ERY concentrations of 2.0 mg L^-1; pH 8.0 and percent of NaCl 10% w/v](image)

**Effect of temperature**

The effect of temperature on the extraction of ERY solution was investigated at pH 8.0 while a extraction time of 5.0 min was performed. The results showed that the extraction efficiency of the ERY as a function of temperature in the range of 25-60° C was not significantly affected by temperature.

**Effect of salt in aqueous sample**

According som researchers, the addition of salt to the samples has been advantageouse for the extraction efficiency of many compounds in SPME (37). Therefore, influence of salt addition in the range 0–20 % (w/v) of NaCl was considered on extraction efficiency of the ERY. The results are demonstrated in Fig 9. The extraction efficiency increased for ERY at salt concentration below 10% (w/v), resulting, presumably, from the salting-out effect. However, apart from the salting-out effect, the presence of salt can change the physical properties of the extraction

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film in the interface of aqueous feed solution and sorbent. It may lead to change of the aqueous activity coefficient of analytes, thus increasing the diffusion rates of the analytes into the sorbent. The extraction efficiency decreased slightly for solution with 15% (w/v) salt. Somewhat surprisingly, extraction efficiency decreased with increasing salt concentration for ERY. The effect of NaCl on the extraction of ERY is probably due to salting-out effect, which decreases the solubility of the analytes and thus increases their partition [38] into absorbent. So, further extractions were carried out at addition of 15% (w/v) salt.

Calibration curve and real samples analysis

Under the selected experimental conditions (pH 8, Fe₃O₄ amount of 10.0 mg, extraction time of 5.0 min, percent of NaCl 10% w/v) the erythromycin concentration varied with different concentrations. The linear dependencies were established between the slopes of the adsorption curves and the erythromycin concentration (fig 10). Applicability of the purposed method to real samples was apprised by extraction and determination of spiked ERY in tap water (dezfulcity, Iran) as reported in Table 1.

CONCLUSION

It is distinct from the data obtained in this research that Fe₃O₄ nanoparticles are adsorbents for ERY antibiotics in water sample. Treatment of Fe₃O₄ nanoparticles and the extraction and determination of the ERY in water samples were investigated. This present method is a fast, very easy and inexpensive one that does not need any especial equipment and can be used in every laboratories.

This method can be used in environmental pollution control organizations, pharmacy companies and clinical diagnosis laboratories.

REFERENCES


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